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Identifying bracovirus and ichnovirus genes involved in virion morphogenesis

Ange Lorenzi[1], Michael R Strand[1], Gaelen R Burke[1] and Anne-Nathalie Volkoff[2]

Bracoviruses (BVs) and ichnoviruses (IVs) evolved from different endogenized viruses but through convergence have been coopted by parasitoids in the families Braconidae and Ichneumonidae for similar functions in parasitizing hosts. Experimentally studying the role of endogenized viral genes in virion morphogenesis remains a key challenge in the study of BVs and IVs. Here we summarize how multiomics, electron microscopy, and RNA interference (RNAi) methods have provided new insights about BV and IV gene function.

Addresses
[1] Department of Entomology, University of Georgia, Athens 30602, GA, USA

[2] UMR DGIMI, University of Montpellier, INRAE, Montpellier 34 095, France

Corresponding authors: Lorenzi, Ange (ange.lorenzi@uga.edu), Volkoff, Anne-Nathalie (anne-nathalie.volkoff@inrae.fr)

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Introduction

As summarized in other contributions to this issue, several thousand parasitoid wasps in the family Braconidae produce bracoviruses (BVs) while several thousand other parasitoids in the family Ichneumonidae produce ichnoviruses (IVs) [1–4]. BVs and IVs have historically been referred to as polydnaviruses because both package multiple circular, double-stranded DNAs that encode virulence genes [5]. These DNAs are integrated into the germline of the wasp but are amplified, processed and packaged into virions in a specific ovarian tissue of female wasps named the calyx. The genes required to produce virions are also integrated into the germline of wasps [1–4]. These genes are solely expressed in calyx cells where BV and IV virions assemble [6••,7••,8], but none reside on the virulence-gene containing DNAs that are packaged into virions [7••,9–11]. Females inject eggs concurrently with BVs or IVs into hosts, which primarily are larval-stage Lepidoptera (moths and butterflies). After infecting different cell types, virulence genes on the viral genome segments in virions are expressed, which alter host immune defenses and growth in ways that enable wasp progeny to develop [1,2]. In turn, wasp offspring survival enables BV and IV genome components to persist via vertical transmission.

Despite sharing many functional features, comparative genomic data unambiguously indicate BVs and IVs evolved from different ancestors. A virus in the family *Nudiviridae* integrated ~100 million years ago into the germline of the braconid that gave rise to BV-producing wasps, while a different type of virus that remains undiscovered or is extinct likely integrated into the germline of two ichneumonid ancestors that gave rise to IV-producing wasps [6••,7••,12,13•,14]. Thus, convergent evolution underlies braconids and ichneumonids similarly repurposing different endogenized viruses to produce virions that transfer virulence genes to hosts. Virulence genes from a number of BV and IV producing wasp species have been characterized, with previous reviews summarizing their origins and functions [1,3,15]. In contrast, only recently has progress been made toward identifying the genes required to produce BVs and IVs in calyx cells, which we summarize here.

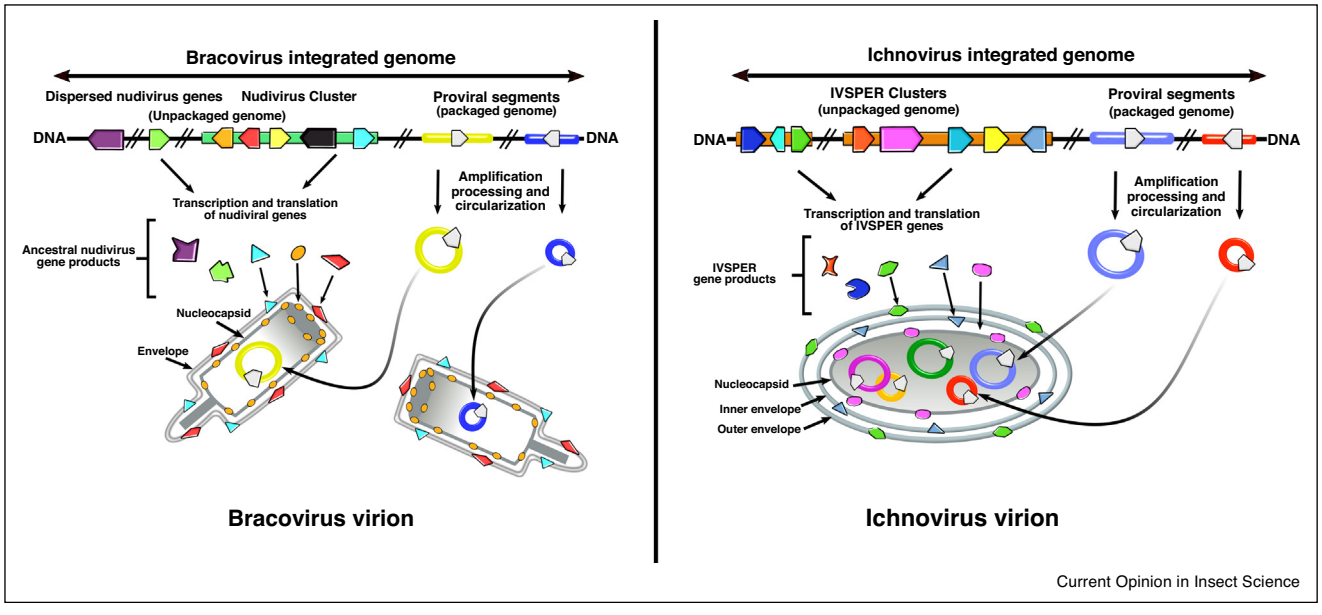
BV and IV gene inventories

The genome components acquired from the virus ancestors of BVs and IVs had to be identified before any functional studies could be initiated. Several wasp species have been studied in this regard, but most information derives from whole genome sequencing of two BV-producing braconids from the subfamily Microgastrinae (*Cotesia congregata* and *Microplitis demolitor*) [16••,17], whole genome sequencing of two IV-producing ichneumonids from the subfamily Campopleginae (*Hyposoter didymator* and *Campopletis sonorensis*) [18•], and partial sequence data for an IV-producing ichneumonid in the subfamily Banchinae (*Glypta fumiferanae*) [13•]. Resulting data could then be coupled to: 1) transcriptome analysis of wasp ovaries when virions are forming in calyx cells and 2) proteomic analysis of virions to identify candidate genes with potential functions in virion morphogenesis [6••,7••,8,13•,18•,19,20••,21••].

In the case of BVs, sequence analysis of *C. congregata* and *M. demolitor* identified many genes that shared recognizable homology with genes in one or more nudiviruses. Approximately half of these genes reside in a 100 kb domain named the nudivirus cluster that is nearly identical in architecture between *C. congregata* and *M. demolitor*, while the remainder are broadly dispersed to largely different locations in the genomes of these two wasp species [16••,17,22] (Figure 1). Most of the nudivirus genes are only expressed in wasp ovaries when virions are being produced, including a number that encode proteins present in virions, which consist of one or more cylindrical nucleocapsids surrounded by a single envelope [6••,8] (Figure 1). A total of 33 core genes are shared among all nudiviruses that have been sequenced but none have been functionally analyzed [23–25]. However, the *Nudiviridae* is also sister to the *Baculoviridae* in which all sequenced species in both families share 21 core genes. All of these shared core genes have been studied in model baculoviruses, which has resulted in identified functions for each in genome replication, transcription or virion formation while also lending predictions for their functions in nudiviruses [26]. Comparing the inventory of nudivirus genes that have been identified in *C. congregata* and *M. demolitor* further indicates both encode 18 of the 21 core genes that are shared between nudiviruses and baculoviruses [17,18•,22]. These include four genes whose products form the DNA-dependent RNA polymerase that specifically transcribes virion structural genes. Importantly though, nudiviruses and BVs also encode many other genes that are unknown from baculoviruses. The virulence gene-containing DNAs that are amplified, processed, and packaged into BV virions reside in the genomes *C. congregata* and *M. demolitor* as proviral segments (Figure 1) [27,28]. Many BV proviral segments are tandemly arrayed and form macroloci [10,16••,17]. Flanking sequences on BV proviral segments also share features that suggest they originated from the nudivirus ancestor although many of the virulence genes derive from other sources including wasps, other insects or other eukaryotes [15,29–31].

In the case of IVs, studies that initially focused on *H. didymator* identified several intronless genes in the wasp genome whose products are present in virions [7••]. None are homologs of genes in any known virus although some

Figure 1



BV and IV genome components consist of genes with functions in producing virions and proviral segments. For the BVs produced by *C. congregata* and *M. demolitor*, some genes inherited from the nudivirus ancestor reside in a domain named the nudivirus cluster (green bar with genes indicated as broad orange, red, yellow, black and blue arrows) while other genes inherited from the nudivirus ancestor are dispersed elsewhere in the wasp genome (broad purple and light green arrows) (left upper panel). Proviral segments containing virulence genes (yellow and blue bars with gray arrows) are also integrated in the wasp genome (left upper panel). Double-hash marks indicate different regions of the wasp genome. Several nudivirus gene products are virion structural proteins while others regulate transcription and translation of the structural genes (left lower panel). Proviral segments are amplified and processed in calyx cells at the same time nudivirus genes are being expressed, which results in circular double-stranded DNA segments that are packaged into cylindrical, tailed nucleocapsids with one envelope (left lower panel). For the IVs produced by *H. didymator* and *C. sonorensis*, genes in domains called IVSPERs (orange bars) are expressed in calyx cells and produce proteins detected in virions (right upper and lower panels). Proviral segments containing virulence genes (violet and red bars) reside elsewhere in the genomes of wasps (right upper panel). Proviral segments are amplified and processed in calyx cells to produce circular, double-stranded DNAs that are packaged into a lenticular nucleocapsid that upon maturity is surrounded by two envelopes (right upper and lower panels).

contain domains shared with genes from some nucleocytoplasmic large DNA viruses (NCLDVs) [13•]. Mapping these genes to the *H. didymator* genome indicates all reside in clusters named 'Ichnovirus Structural Protein Encoding Regions' (IVSPERs) (Figure 1). IVSPER organizational features strongly suggest they derive from an endogenized virus [7••]. Like nudivirus genes in braconids, genes in IVSPERS are specifically expressed in ichneumonid ovaries when virions, which each consist of a lenticular nucleocapsid and two envelopes, are being produced (Figure 1) [32]. The DNAs containing virulence genes that are packaged in IV virions are also integrated in the genomes of wasps as proviral segments [18•]. Proviral segments and IVSPERs also share a gene family, named N-genes, which provides another line of evidence that IVSPERs and proviral segments both derive from a virus ancestor [7••,18•]. Gene content and order for the IVSPERs in *H. didymator* are largely shared with *C. sonorensis*, whereas gene content and the location of proviral segments differs considerably [18•]. IV proviral segments are also much more dispersed when compared to BV proviral segments [18•]. Thus, several lines of evidence implicate IVSPER genes in virion formation, but the lack of homology to known genes results in sequence data alone providing few or no functional predictions.

BV and IV morphogenesis

The second piece of essential information needed to identify genes with functions in producing BVs and IVs is comparative data on virion morphogenesis. Several studies using electron microscopy (EM) provide information on how BV and IV virions assemble. Calyx cells reside in a specialized region at the juncture between the paired ovaries and lateral oviducts (Figure 2). EM studies combined with transcriptome analyses indicate that BV and IV replication both begin early in the wasp pupal stage [6••,8,21••,33,34••]. BV and IV virions also both assemble in calyx cell nuclei. As previously noted, BV and IV virions morphologically differ from one another. Several steps associated with morphogenesis and virion release from calyx cells also differ.

BV morphogenesis begins with *de novo* formation of rounded envelopes followed by the appearance of cylindrical nucleocapsids in proximity to virogenic stroma. Foci of nucleocapsids acquiring envelopes thereafter appear followed by nuclei greatly enlarging, and virions consisting of a nucleocapsid surrounded by an elongated envelope assembling into parallel arrays. Thereafter virions progressively become disordered, but near fully fill the nucleus before being released into the lumen of the lateral oviduct by successive lysis of the nuclear envelope and the plasma membrane (Figure 2) [21••,35].

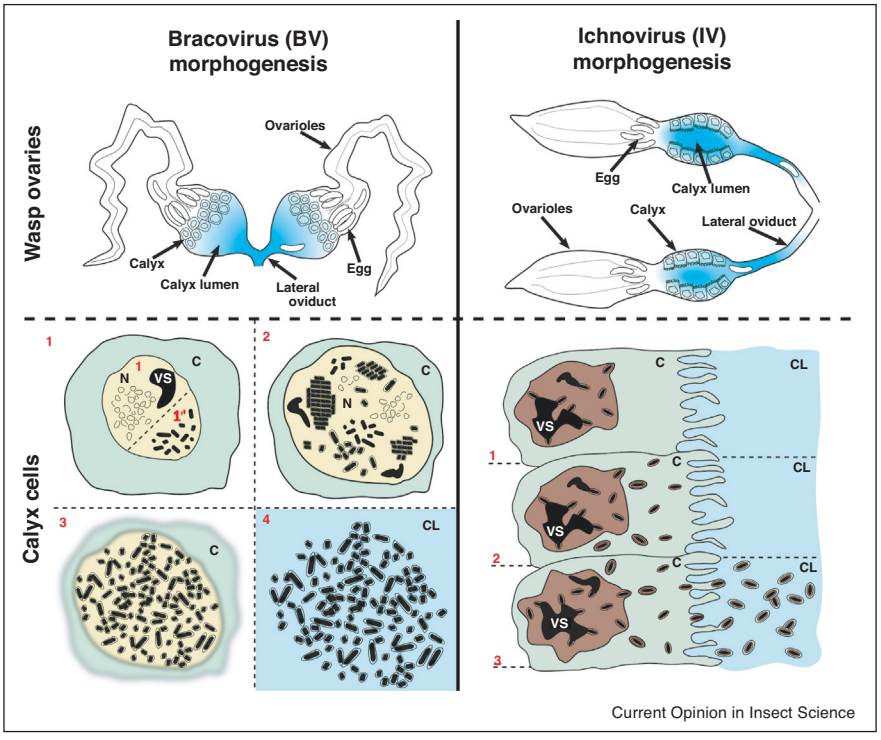
IV morphogenesis in campoplegine ichneumonids begins with the concurrent *de novo* formation of lenticular nucleocapsids and envelopes at the periphery of an electron dense virogenic stroma (Figure 2). Individual nucleocapsids are then surrounded by an envelope to produce a subvirion. Subvirions bud through the nuclear envelope to reach the cytoplasm of calyx cells. At this step subvirions are transiently surrounded by nuclear membranes, which are lost during migration within the cytoplasm to the apical region of the calyx cell. Subvirions then bud through the plasma membrane where they acquire a second envelope to become mature virions that accumulate in the calyx lumen (Figure 2) [32,36]. IV morphogenesis in banchine ichneumonids begins similarly in the nuclei of calyx cells, but notably differs from campoplegines in regard to several, smaller, rod-shaped nucleocapsids being surrounded by an envelope to produce a subvirion [13•]. Thereafter though, the subvirions produced in banchines also bud through the nuclear envelope, migrate through the cytoplasm and exit calyx cells by budding through the plasma membrane where they acquire a second envelope to produce a mature virion [13•].

RNA interference (RNAi) as a tool for identifying genes with functions in BV and IV morphogenesis

The third area of need was to develop approaches that could be used to manipulate BV and IV genes so that their functions in virion formation could be studied experimentally. This required different approaches from those used with baculoviruses because BVs and IVs do not package their replication machinery into virions, which prevents using cell lines and recombination-based methods to genetically manipulate genes of interest. Instead, what has primarily been used is RNA interference (RNAi), which has been shown to reduce transcript abundance of particular BV and IV genes that are expressed in calyx cells with high efficacy.

The first studies using RNAi to knock down BV genes in wasps were conducted in *M. demolitor*, which produces *M. demolitor* bracovirus (MdBV) [20••]. Initial RNAi assays deliberately targeted core genes shared with baculoviruses to test the prediction that their functions in replication and/or morphogenesis remained conserved. Results showed that knockdown of two core genes encoding subunits of the RNA polymerase (*lef-4* and *lef-9*) disabled expression of several structural core genes that in baculoviruses are known to be specifically transcribed by the viral RNA polymerase [16••,20••]. Knockdown of other baculovirus core gene homologs also resulted in phenotypes that were consistent with retaining conserved functions [20••]. With evidence that several genes acquired from the nudivirus ancestor that are shared with baculoviruses exhibit conserved functions, RNAi was next used to study the function of other nudivirus genes in *M. demolitor* that are unknown from baculoviruses. In this regard, all nudiviruses encode two predicted

Figure 2

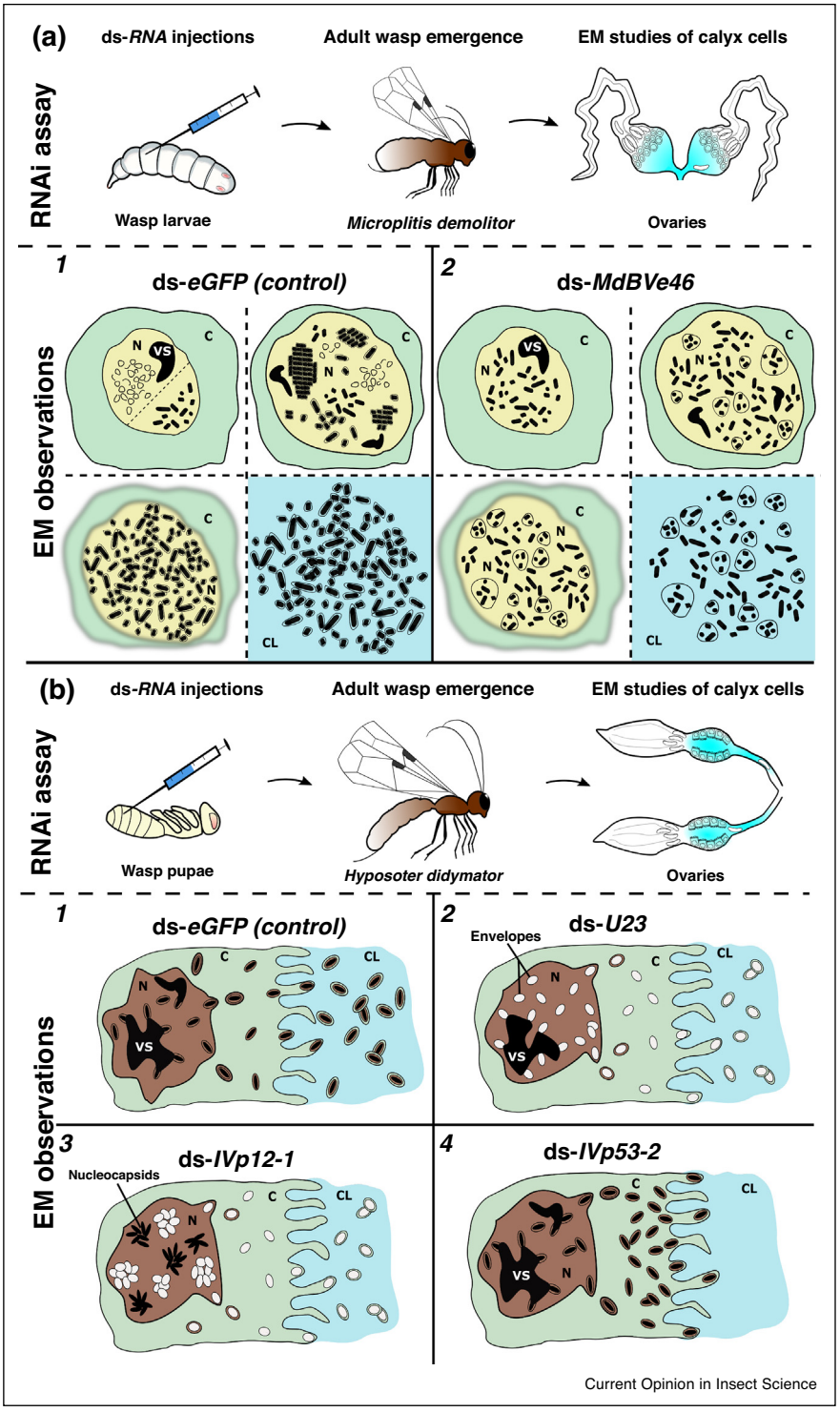


Schematic illustrating bracovirus (BV) and ichnovirus (IV) morphogenesis. The upper panels show the ovaries of a braconid, microgastrine like *M. demolitor* (left) and ichneumonid campoplegine like *H. didymator* (right) with locations of the ovarioles, eggs, calyx cells, calyx lumen and lateral oviducts indicated. The lower panels show the progression of BV (left) and IV (right) virion formation. For BVs, (1) envelopes form *de novo* in the rounded nucleus (N) of a calyx cell, which is surrounded by cytoplasm (C). Envelopes usually appear in proximity to virogenic stroma (VS), which is followed (1′) by the *de novo* appearance of nucleocapsids (black cylinders) in domains that are adjacent to where envelopes form. (2) Individual nucleocapsids are surrounded by one envelope to form virions that accumulate in parallel arrays. (3) Virions become disordered while near fully filling the nucleus, which is followed by calyx cell lysis. (4) Calyx cell lysis releases virions into the calyx lumen (CL). For IVs, (1) envelopes and lenticular nucleocapsids concurrently form *de novo* at the periphery of virogenic stroma. (2) Individual nucleocapsids are surrounded by one envelope to form subvirions that bud through the nuclear envelope into the cytoplasm. (3) Subvirions acquire a second envelope by budding through the plasma membrane to produce mature virions that accumulate in the calyx lumen.

recombinases with *vlf-1* being a homolog of a baculovirus gene and *int* being unknown from baculoviruses [20••]. Knockdown of MdBV *vlf-1* and *int* family members suggests that both are involved in processing proviral segments after amplification into the circular DNAs that are packaged into virions [20••]. Results also implicate particular sequences in proviral segments as recombinase binding sites, which suggests these sequences also derive from the nudivirus ancestor. By separating MdBV virions into nucleocapsid and envelope fractions, proteomic data analysis identified several other genes present in nudiviruses but unknown in baculoviruses as candidate envelope or nucleocapsid structural proteins [21••]. One of these genes, named *MdBVe46*, is a homolog of a nudivirus gene of unknown function named *HzNVorf-64* whose product localizes to the MdBV envelope fraction [21••]. EM analysis and immunoblotting experiments confirm that *MdBVe46* encodes an envelope protein while knockdown of this gene by RNAi results in severe defects in envelope formation (Figure 3a) [21••]. Knockdown of *MdBVe46* also greatly reduces the ability of MdBV to infect host cells, which is required for expression of virulence gene products that protect *M. demolitor* larvae from the host's immune system [21••].

In the case of IVs, none of the genes in IVSPERs are homologs of any known genes but RNAi assays combined with EM and immunolabeling studies recently provided important insights into the function of six genes in morphogenesis and trafficking of *H. didymator* ichnovirus (HdIV) (Figure 3b) [34••]. The first, named *IVp12-1*, encodes a protein that co-localizes with the virogenic stroma and the nuclear subvirion. RNAi knockdown of this gene results in nucleocapsids that remain trapped in the nucleus with only empty envelopes budding from calyx cells (Figure 3b). Knockdown of *U23* and *IVSP4-1* indicate that both are required for nucleocapsid formation as loss of each result in no nucleocapsids forming in calyx

Figure 3


(a)
ds-*RNA* injections
Adult wasp emergence
EM studies of calyx cells
RNAi assay
Wasp larvae
Microplitis demolitor
Ovaries
EM observations
1
ds-*eGFP (control)*
2
ds-*MdBVe46*
N
VS
C
CL
(b)
ds-*RNA* injections
Adult wasp emergence
EM studies of calyx cells
RNAi assay
Wasp pupae
Hyposoter didymator
Ovaries
EM observations
1
ds-*eGFP (control)*
2
ds-*U23*
Envelopes
3
ds-*IVp12-1*
Nucleocapsids
4
ds-*IVp53-2*
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(Figure 3 Legend) Schematic illustrating RNAi assays and phenotypes associated with knockdown of *MdBVe46* in *M. demolitor* and several genes in IVSPERs in *H. didymator*. **(a)** ds-*RNA* is injected into a last instar *M. demolitor* larva, which is followed by emergence as an adult wasp and collection of the paired ovaries for EM analysis. 1. Wasps injected with ds-*eGFP* (negative control) results in no reduction in *MdBVe46* transcript abundance while virion morphogenesis progresses similarly to non-injected wasps with all virions consisting of a single nucleocapsid surrounded by one envelope (see Figure 2, left panel). 2. Wasps injected with ds-*MdBVe46* results in significant reduction of *MdBVe46* and a phenotype in which nucleocapsids (black cylinders) but few envelopes form in calyx cells. Resulting virions either lack envelopes or an envelope surrounds multiple nucleocapsids, which is followed by calyx cell lysis that releases defective virions into the calyx lumen. **(b)** ds-*RNA* injected into 1-day old *H. didymator* pupae, which is followed by emergence as an adult wasp and collection of the paired ovaries for EM analysis. 1. Wasps injected with ds-*eGFP* (negative control) results in virion morphogenesis proceeding similarly to non-injected wasps with each nucleocapsid (black elipsoid) being surrounded by envelopes after budding from a calyx cell into the calyx lumen (see Figure 2, right panel). 2. Injection of ds-*U23* into wasps results in significant knockdown of *U23* and a phenotype in which envelopes (white ellipsoids) but no nucleocapsids form followed by envelopes budding into the calyx lumen to form double-enveloped vesicles. 3. Injection of ds-*IVp12-1* results in significant knockdown of *IVp12-1* and a phenotype in which nucleocapsids (black elipsoids) and envelopes (white elipsoids) assemble separately in calyx cell nuclei. Aggregates of nucleocapsids remain in the nucleus while envelopes accumulate in the calyx lumen. 4. Injection of ds-*IVp53-2* results in significant knockdown of *IVp53-2* and a phenotype in which virions accumulate in the cytoplasm of calyx cells rather than budding into the calyx lumen. C: cytoplasm, CL: calyx lumen, N: nucleus, VS: virogenic stroma.

cell nuclei (Figure 3b) [34••]. Antibody labeling studies further support these findings by showing that U23 is a structural protein that localizes to nucleocapsids. Knockdown of two other genes, *IVSP3-1* and *U22*, also results in abnormal accumulation of subvirions lacking nucleocapsids in calyx cell nuclei, which suggests roles for both in the passage of the nuclear envelope to the cytoplasm [34••]. Lastly, immunolabeling studies show that IVp53-2 localizes to the HdIV outer envelope. Knockdown of *IVp53-2* disables the budding process through the plasma membrane and results in subvirions being trapped in calyx cell cytoplasm (Figure 3b), suggesting that IVp53-2 is required for budding through the plasma membrane and formation of mature virions [34••].

Conclusions and future needs

The preceding summary illustrates how multiomic data together with functional assays can be used to characterize BV and IV genes with functions in virion morphogenesis. A number of other nudivirus genes in BV-producing braconids and IVSPER genes in IV-producing ichneumonids have been identified but their functions remain unknown. However, most are likely amenable to study using similar methods to those discussed here. Different endogenized viruses have also recently been identified in other lineages of parasitoid wasps that produce virus-like particles (VLPs) [14,22,37–40]. Methods developed in *M. demolitor* and *H. didymator* could likely be adapted to characterize the function of these viral genes in VLP formation. In terms of future challenges, we see three that are especially important. First, current studies on BVs rely on homology to known nudivirus genes, which means that other genes required to produce BVs, whether from the endogenized viral ancestor or from the wasp, may remain unrecognized and also could have important functions in viral replication. Analogously, some genes outside of IVSPERs could have functions in producing IVs. Possible strategies to begin identifying these factors include further proteomic analysis of BV and IV virions with emphasis on genes that are not nudivirus homologs (BVs) or that map outside of IVSPERs (IVs). Genes of interest could then be further characterized as discussed in this review. Second, there is a need to understand why the genes required to produce BVs and IVs are only expressed in calyx cells. In the case of BVs, these studies likely need to focus on the factors responsible for expression of the nudivirus RNA polymerase in calyx cells given evidence that this enzyme transcribes most if not all of the virion structural genes. Third, while RNAi provides an approach for conducting loss of function assays, alternative methods or approaches for conducting gain of function experiments are currently not possible. CRISPR-Cas mediated approaches to alter the structure or function of endogenized viral genes would be of value, but injection of early-stage wasp eggs presents significant challenges due to their small size as does downstream development, which currently depends on maturation inside a host to produce progeny. However, other approaches for introducing CRISPR-Cas components into oocytes have been developed that could be of value in studying BV and IV-producing wasps [41,42].

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Strand MR, Burke GR: **Polydnaviruses: nature's genetic engineers**. *Annu Rev Virol* 2014, **1**:333-354.

2. Gauthier J, Drezen J-M, Herniou EA: **The recurrent domestication of viruses: major evolutionary transitions in parasitic wasps**. *Parasitology* 2018, **145**:713-723.

3. Darboux I, Cusson M, Volkoff A-N: **The dual life of ichnoviruses**. *Curr Opin Insect Sci* 2019, **32**:47-53.

4. Strand MR, Burke GR: **Polydnaviruses: evolution and function**. *Curr Issues Mol Biol* 2020, **34**:163-182.

5. Stoltz DB, Krell P, Summers MD, Vinson SB: **Polydnaviridae - a proposed family of insect viruses with segmented, double-stranded, circular DNA genomes**. *Intervirology* 1984, **21**:1-4.

6. •• Bézier A, Annaheim M, Herbinière J, Wetterwald C, Gyapay G, Bernard-Samain S, Wincker P, Roditi I, Heller M, Belghazi M *et al.*: **Polydnaviruses of braconid wasps derive from an ancestral nudivirus**. *Science* 2009, **323**:926-930
Presents the first evidence that BVs evolved from an ancestral nudivirus.

7. •• Volkoff A-N, Jouan V, Urbach S, Samain S, Bergoin M, Wincker P, Demettre E, Cousserans F, Provost B, Coulibaly F *et al.*: **Analysis of virion structural components reveals vestiges of the ancestral ichnovirus genome**. *PLoS Pathog* 2010, **6**:e1000923
Presents the first evidence that proteins present in IV virions reside in domains of the wasp genome named IVSPERs which have organizational features that suggest they derive from an endogenized virus.

8. Burke GR, Strand MR: **Deep sequencing identifies viral and wasp genes with potential roles in replication of *Microplitis demolitor* Bracovirus**. *J Virol* 2012, **86**:3293-3306.

9. Espagne E, Dupuy C, Huguet E, Cattolico L, Provost B, Martins N, Poirié M, Periquet G, Drezen JM: **Genome sequence of a polydnavirus: insights into symbiotic virus evolution**. *Science* 2004, **306**:286-289.

10. Desjardins CA, Gundersen-Rindal DE, Hostetler JB, Tallon LJ, Fuester RW, Schatz MC, Pedroni MJ, Fadrosh DW, Haas BJ, Toms BS *et al.*: **Structure and evolution of a proviral locus of *Glyptapanteles indiensis* bracovirus**. *BMC Microbiol* 2007, **7**:61.

11. Webb BA, Strand MR, Dickey SE, Beck MH, Hilgarth RS, Barney WE, Kadash K, Kroemer JA, Lindstrom KG, Rattanadechakul W *et al.*: **Polydnavirus genomes reflect their dual roles as mutualists and pathogens**. *Virology* 2006, **347**:160-174.

12. Murphy N, Banks JC, Whitfield JB, Austin AD: **Phylogeny of the parasitic microgastroid subfamilies (Hymenoptera: Braconidae) based on sequence data from seven genes, with an improved time estimate of the origin of the lineage**. *Mol Phylogenet Evol* 2008, **47**:378-395.

13. • Béliveau C, Cohen A, Stewart D, Periquet G, Djoumad A, Kuhn L, Stoltz D, Boyle B, Volkoff A-N, Herniou EA *et al.*: **Genomic and proteomic analyses indicate that banchine and campoplegine polydnaviruses have similar, if not identical, viral ancestors**. *J Virol* 2015, **89**:8909-8921
Results indicate that IVs produced by wasps in the subfamily Banchinae derive from a similar ancestor as IVs produced by wasps in the subfamily Campopleginae.

14. Burke GR, Hines HM, Sharanowski BJ: **The presence of ancient core genes reveals endogenization from diverse viral ancestors in parasitoid wasps**. *Genome Biol Evol* 2021, **13**: evab105.

15. Huguet E, Serbielle C, Moreau SJM: **Evolution and origin of polydnavirus virulence genes**. *Parasitoid Viruses*. Elsevier; 2012:63-78.

16. •• Burke GR, Walden KKO, Whitfield JB, Robertson HM, Strand MR: **Widespread genome reorganization of an obligate virus mutualist**. *PLoS Genet* 2014, **10**:e1004660
First genome of a BV-producing wasp showing that the ancestral nudivirus genome is dispersed.

17. Gauthier J, Boulain H, van Vugt JJFA, Baudry L, Persyn E, Aury J-M, Noel B, Bretaudeau A, Legeai F, Warris S *et al.*: **Chromosomal scale assembly of parasitic wasp genome reveals symbiotic virus colonization**. *Commun Biol* 2021, **4**:104.

18. • Legeai F, Santos BF, Robin S, Bretaudeau A, Dikow RB, Lemaitre C, Jouan V, Ravallec M, Drezen J-M, Tagu D *et al.*: **Genomic architecture of endogenous ichnoviruses reveals distinct evolutionary pathways leading to virus domestication in parasitic wasps**. *BMC Biol* 2020, **18**:89
Results show that IVSPERs in two IV-producing species in the subfamily Campoplegine exhibit conserved gene order and location in wasp genomes.

19. Wetterwald C, Roth T, Kaeslin M, Annaheim M, Wespi G, Heller M, Mäser P, Roditi I, Pfister-Wilhelm R, Bézier A *et al.*: **Identification of bracovirus particle proteins and analysis of their transcript levels at the stage of virion formation**. *J Gen Virol* 2010, **91**:2610-2619.

20. •• Burke GR, Thomas SA, Eum JH, Strand MR: **Mutualistic polydnaviruses share essential replication gene functions with pathogenic ancestors**. *PLoS Pathog* 2013, **9**:e1003348
Reports the first functional evidence that several nudivirus genes are required for BV formation.

21. •• Arvin MJ, Lorenzi A, Burke GR, Strand MR: **MdBVe46 is an envelope protein that is required for virion formation by *Microplitis demolitor* bracovirus**. *J Gen Virol* 2021, **102**:001565
First proteomic study of BVs that separated virions into an envelope and nucleocapsid fraction, which facilitated functional analysis of a previously uncharacterized nudivirus gene that is required for envelope formation.

22. Burke GR, Walden KKO, Whitfield JB, Robertson HM, Strand MR: **Whole genome sequence of the parasitoid wasp *Microplitis demolitor* that harbors an endogenous virus mutualist**. *G3* 2018, **30**:2875-2880.

23. Wang Y, Bininda-Emonds ORP, Jehle JA: **Nudivirus genomics and phylogeny**. *Viral Genomes - Molecular Structure, Diversity, Gene Expression Mechanisms and Host-Virus Interactions*. InTech; 2012.

24. Thézé J, Bézier A, Periquet G, Drezen J-M, Herniou EA: **Paleozoic origin of insect large dsDNA viruses**. *Proc Natl Acad Sci U S A* 2011, **108**:15931-15935.

25. Harrison RL, Herniou EA, Bézier A, Jehle JA, Burand JP, Theilmann DA, Krell PJ, van Oers MM, Nakai M: **ICTV report consortium: ICTV virus taxonomy profile: Nudiviridae**. *J Gen Virol* 2020, **101**:3-4.

26. Rohrmann GF: *Baculovirus Molecular Biology*. edn 4. Bethesda (MD): National Center for Biotechnology Information (US); 2019.

27. Louis F, Bézier A, Periquet G, Ferras C, Drezen J-M, Dupuy C: **The bracovirus genome of the parasitoid wasp *Cotesia congregata* is amplified within 13 replication units, including sequences not packaged in the particles**. *J Virol* 2013, **87**:9649-9660.

28. Burke GR, Simmonds TJ, Thomas SA, Strand MR: ***Microplitis demolitor* bracovirus proviral loci and clustered replication genes exhibit distinct DNA amplification patterns during replication**. *J Virol* 2015, **89**:9511-9523.

29. Drezen J-M, Bézier A, Lesobre J, Huguet E, Cattolico L, Periquet G, Dupuy C: **The few virus-like genes of *Cotesia congregata* bracovirus**. *Arch Insect Biochem Physiol* 2006, **61**:110-122.

30. Desjardins CA, Gundersen-Rindal DE, Hostetler JB, Tallon LJ, Fadrosh DW, Fuester RW, Pedroni MJ, Haas BJ, Schatz MC, Jones KM *et al.*: **Comparative genomics of mutualistic viruses of *Glyptapanteles* parasitic wasps**. *Genome Biol* 2008, **9**:R183.

31. Herniou EA, Huguet E, Thézé J, Bézier A, Periquet G, Drezen J-M: **When parasitic wasps hijacked viruses: genomic and functional evolution of polydnaviruses**. *Philos Trans R Soc Lond B Biol Sci* 2013, **368**:20130051.

32. Volkoff A-N, Ravallec M, Bossy J-P, Cerutti P, Rocher J, Cerutti M, Devauchelle G: **The replication of *Hyposoter didymator* polydnavirus: cytopathology of the calyx cells in the parasitoid**. *Biol Cell* 1995, **83**:1-13.

33. Stoltz DB, Vinson SB: **Viruses and parasitism in insects**. *Adv Virus Res* 1979, **24**:125-171.

34. •• Lorenzi A, Ravallec M, Eychenne M, Jouan V, Robin S, Darboux I, Legeai F, Gosselin-Grenet A-S, Sicard M, Stoltz D *et al.*: **RNA interference identifies domesticated viral genes involved in assembly and trafficking of virus-derived particles in ichneumonid wasps**. *PLoS Pathog* 2019, **15**:e1008210
Provides the first functional evidence that several genes in IVSPERs are required for IV formation.

35. Wyler T, Lanzrein B: **Ovary development and polydnavirus morphogenesis in the parasitic wasp *Chelonus inanitus*. II. Ultrastructural analysis of calyx cell development, virion formation and release**. *J Gen Virol* 2003, **84**:1151-1163.

36. Djoumad A, Stoltz D, Béliveau C, Boyle B, Kuhn L, Cusson M: **Ultrastructural and genomic characterization of a second banchine polydnavirus confirms the existence of shared features within this ichnovirus lineage**. *J Gen Virol* 2013, **94**:1888-1895.

37. Pichon A, Bézier A, Urbach S, Aury J-M, Jouan V, Ravallec M, Guy J, Cousserans F, Thézé J, Gauthier J *et al.*: **Recurrent DNA virus domestication leading to different parasite virulence strategies**. *Sci Adv* 2015, **1**:e1501150.

38. Lepetit D, Gillet B, Hughes S, Kraaijeveld K, Varaldi J: **Genome sequencing of the behavior manipulating virus LbFV reveals a possible new virus family**. *Genome Biol Evol* 2016, **8**:3718-3739.

39. Di Giovanni D, Lepetit D, Guinet B, Bennetot B, Boulesteix M, Couté Y, Bouchez O, Ravallec M, Varaldi J: **A behavior-manipulating virus relative as a source of adaptive genes for *Drosophila* parasitoids**. *Mol Biol Evol* 2020, **37**:2791-2807.

40. Burke GR, Simmonds TJ, Sharanowski BJ, Geib SM: **Rapid viral symbiogenesis via changes in parasitoid wasp genome architecture**. *Mol Biol Evol* 2018, **35**:2463-2474.

41. Chaverra-Rodriguez D, Macias VM, Hughes GL, Pujhari S, Suzuki Y, Peterson DR, Kim D, McKeand S, Rasgon JL: **Targeted delivery of CRISPR-Cas9 ribonucleoprotein into arthropod ovaries for heritable germline gene editing**. *Nat Commun* 2018, **9**:3008.

42. Chaverra-Rodriguez D, Benetta ED, Heu CC, Rasgon JL, Ferree PM, Akbari OS: **Germline mutagenesis of *Nasonia vitripennis* through ovarian delivery of CRISPR-Cas9 ribonucleoprotein**. *Insect Mol Biol* 2020, **29**:569-577.